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Replication Patterns of Multiple Plasmids Coexisting in *Escherichia coli*

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The replication patterns of several plasmids were measured simultaneously during the cell division cycle of *Escherichia coli* B/r. F plasmids harboring *oriS*, both *oriS* and *oriV*, pSC101, and pBR322 were found to replicate at all stages of the cell division cycle with kinetics which were indistinguishable from one another and clearly different from the periodic synthesis of the minichromosomes pAL49 and pAL70.

Bacterial plasmids have become popular model systems for studies of the regulation of DNA synthesis because they replicate autonomously in the cell but are dispensable for cell viability (21, 26). The possibility of a linkage between the plasmid-borne regulatory system and either chromosome replication or an aspect of the cell division cycle has been of interest, particularly for plasmids which are present at low copy numbers and yet are stably inherited (16, 21). Replication of such plasmids was initially thought to be restricted to a limited period of the *Escherichia coli* division cycle (5–7, 29), but other work has suggested that this is not the case (1, 4, 9, 23, 24, 28). Recently, however, it has been suggested that the control of mini-F plasmid replication is similar to that of the chromosome when plasmid replication ensues from *oriS*, with *oriV* deleted (27).

The validity of conclusions about the pattern of plasmid replication in the division cycle depends on the ability of the experimental technique to detect a relationship between replication and the cycle if such a relationship exists. Until now, the techniques have been difficult to evaluate because plasmids with known cell cycle-specific replication patterns have not been available to serve as positive experimental controls. This difficulty has been overcome by the discovery that *E. coli* minichromosomes replicate during a discrete interval in the division cycle and by the development of an experimental protocol which allows measurement of the replication of multiple plasmids simultaneously in the same host cell (12, 19). Accordingly, we examined the following plasmid combinations as they replicated together in the same *E. coli* B/r host: (i) the mini-F plasmid pSG21, which replicates solely from *oriS*, pBR322, and minichromosome pAL70, and (ii) F′ *lac* (*oriS oriV*), pSC101, pBR322, and minichromosome pAL49. The replication properties of F plasmid derivatives and pSC101 were of particular interest because, in addition to the very low copy number of F plasmids (24), their maintenance is dependent on the *dnaA* gene product (8, 10, 11, 17), a protein required for initiation of replication from the chromosomal origin of replication.

The membrane elution procedure was used to compare the replication patterns of the F plasmid derivatives and pSC101 with those of minichromosomes pAL49 or pAL70, both of which replicate during a restricted stage in the cell cycle, and pBR322, which replicates in all stages of the cycle (19). For each experiment, 100 ml of minimal salts medium containing glucose (0.1%), Casamino Acids (0.2%; Difco Laboratories, Detroit, Mich.), and kanamycin (100 μg/ml) was inoculated with *E. coli* B/r F26(*thyA his lac*) harboring either pSG21, pBR322 (2), and pAL70 or F′ *lac* (7), pSC101 (3), pBR322, and pAL49 (19). pSG21 is composed of two restriction fragments: (i) a 6,500-base-pair *Hpa*I restriction fragment derived from pDF42 (15), which carries the *oriS* but not the *oriV* replication origin of F, and (ii) a 1,380-base-pair *Pvu*II restriction fragment which harbors an ampicillin resistance determinant. Minichromosome pAL70 (3,772 base pairs) is composed of two *Hae*II restriction fragments which carry the *oriC* region of the chromosome and a chloramphenicol resistance determinant, respectively. pAL70 harbors *oriC* DNA from coordinates −422 to 1030 on the map of Oka et al. (22). Cells were incubated in a shaking water bath at 37°C until the culture contained approximately 5×10^7 cells per ml growing exponentially. The kinetics of plasmid replication during the division cycle was determined by pulse-labeling the exponentially growing culture with 10 μCi of [^{3}H]thymidine per ml (70 to 80 Ci/mmol; Dupont-NEN Products, Boston, Mass.), binding the labeled culture to a nitrocellulose membrane filter (type GS; Millipore Corp., Bedford, Mass.), and measuring the radioactivity in DNAs of each plasmid in newborn cells released continuously from the filter during growth of the filter-bound cells. The newborn cells in the effluent were lysed and prepared for electrophoresis as described previously (19). Portions from individual cell lysate samples were loaded into the wells of a 0.8% agarose slab gel and electrophoresed at 100 V for 18 h in Tris-borate-EDTA buffer (25). In some experiments, each well was loaded with lysate from an equivalent number of newborn cells. Gels were prepared for fluorography (18), and the dried gels were exposed to X-Omat AR X-ray film (Eastman Kodak Co., Rochester, N.Y.) at −70°C for 10 days or fewer. When necessary, individual gels were exposed to film for several different durations to provide clear radioactive images of high- and low-copy-number plasmids in the same gel. The amount of radioactivity incorporated was quantified by densitometric tracing with laser densitometer (LKB Instruments, Inc., Rockville, Md.) of the closed circular plasmid DNA bands in the fluorographs of the gels. Since the closed circular monomers of the plasmids migrated to different positions in the gel because of the size differences, it was possible in a single experiment to compare the replication patterns of each plasmid type.

The fluorograph in Fig. 1 shows the cell cycle replication patterns of pSG21, pBR322, and minichromosome pAL70 after electrophoretic separation of the three plasmids on an

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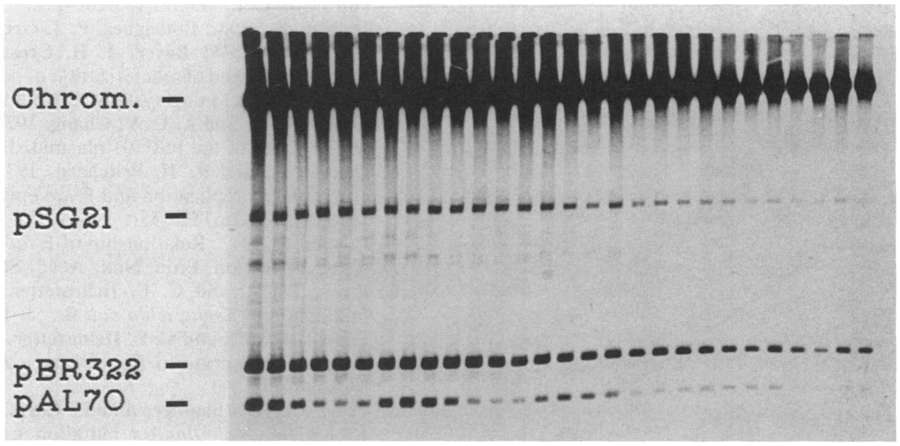

FIG. 1. Fluorograph of radioactive plasmid DNA in newborn cells from the effluent of a membrane filter-bound culture of *E. coli* B/r F26(pSG21, pBR322, pAL70). Cells growing exponentially were pulse-labeled with [^{3}H]thymidine for 4 min, filtered onto the surface of a membrane filter, and eluted with glucose-Casamino Acids minimal medium. Samples of the effluent were collected every 4 min, lysed, and prepared for agarose gel electrophoresis and fluorography. In this experiment, all lanes contained lysate from the same number of newborn cells. Bands corresponding to chromosomal DNA (chrom.) and the closed circular monomers of pSG21, pBR322, and pAL70 are indicated.

agarose gel. It is clear from the fluorograph that it was possible to separate the closed circular forms of the individual plasmid species from one another and from the chromosome. Replication of minichromosome pAL70 exhibited a periodicity which was absent in the other plasmid species. Quantitative comparison of the replication of the three plasmids during the division cycle is shown in Fig. 2. After attachment of the pulse-labeled cells to the membrane filter, the first newborn cells eluted in each generation of growth were progeny of cells at the end of the division cycle at the time of attachment, and the last cells eluted in each generation were progeny of cells at the start of the division cycle. Therefore, the rate of [^{3}H]thymidine incorporation into plasmid DNA as a function of the division cycle is read from right to left in each generation of elution. Of the three plasmids examined, only minichromosome pAL70 replicated during a discrete interval in the division cycle. Replication of plasmids pSG21 and pBR322 was distributed throughout the division cycle at a frequency that appeared to increase gradually during the cycle. The more rapid decrease in radioactive pSG21 DNA per newborn cell during the third generation of elution may be a consequence of either nonrandom plasmid partition into daughter cells (13) or decreased sensitivity of the scanning densitometer to the lightest pSG21 bands on the autoradiograph.

Quantitative comparison of replication during the division cycle of F′ *lac*, pSC101, pBR322, and minichromosome pAL49 is shown in Fig. 3. Periodic synthesis during the division cycle was again observed only for the minichromosome. In all experiments, plasmids pSG21 (*oriS* only), F′ *lac* (*oriS oriV*), pBR322, and pSC101 were capable of replicating at all stages of the division cycle, with no detectable differences in the patterns. This was the case whether all the plasmids were in the same cell, as presented here, or whether only one plasmid was in a cell (data not shown). We have also examined the replication patterns for pML31 (20) and a *Bam*HI deletion derivative of pML31 which replicates solely from *oriS* and found them to be identical to those observed for pSG21 and F′ *lac*.

With this experimental protocol, it has been possible for the first time to analyze the cell cycle replication of a specific plasmid by comparing it with plasmids harbored in the same cell whose replication patterns were known to be either cell cycle specific (minichromosomes) or cell cycle nonspecific (pBR322). The results show that pSG21, F′ *lac*, and pSC101 can replicate at all times during the cell cycle, although a

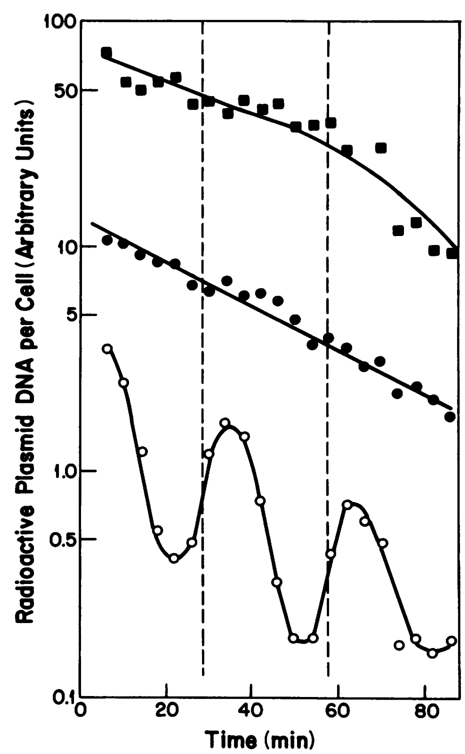

FIG. 2. Radioactive plasmid DNA in newborn cells from the effluent of a membrane filter-bound culture of *E. coli* B/r(pSG21, pBR322, pAL70). Quantitative analysis of the fluorograph presented in Fig. 1 was performed by scanning densitometry. The vertical interrupted lines indicate generations of elution. The radioactivity in the individual plasmids was determined as the absorbance of the closed circular plasmid bands divided by the concentration of cells in the effluent sample. Symbols: ■, pSG21; ●, pBR322; ○, pAL70.

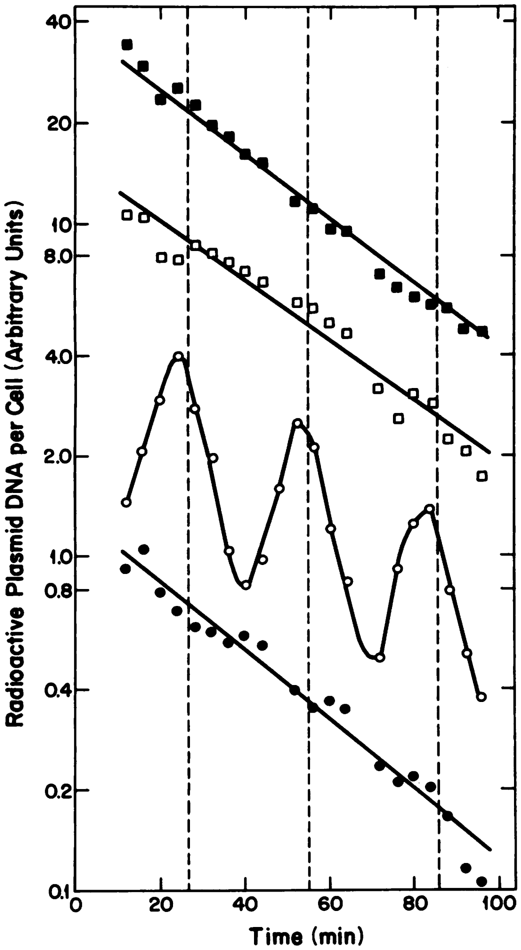

FIG. 3. Radioactive plasmid DNA in newborn cells from the effluent of a membrane filter-bound culture of *E. coli* B/r F26(F′ *lac*, pSC101, pAL49, pBR322). Cells growing exponentially were pulse-labeled and treated as described in the legends to Fig. 1 and 2. Symbols: ■, F′ *lac*; □, pSC101; ○, pAL49; ●, pBR322.

mode of synthesis involving two or more replication windows per cycle might not be detected by this method. Minichromosomes may be unique in their cell cycle-specific mode of replication and for this reason are the best available model system with which to study the replication control mechanism of the chromosome. If there is a relationship between replication and cell division for the lower-copy-number bacterial plasmids, it is probably mediated by a system which can affect cell viability in the absence of plasmid duplication rather than by a system in which an aspect of the division cycle can govern replication (14, 21).

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